Datasheet for ABIN956276

KLH IgM ELISA Kit

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Overview

Quantity:	96 tests
Target:	KLH IgM
Reactivity:	Rat
Method Type:	Sandwich ELISA
Application:	ELISA

Product Details

Sample Type:	Plasma, Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Characteristics:	The Rat Anti-KLH IgM ELISA is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay uses KLH for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated anti-Rat IgM antibodies for detection. Test serum or plasma samples are diluted and incubated in the microtiter wells for 45 minutes. The microtiter wells are subsequently washed and HRP conjugate is added and incubated for 45 minutes. Anti-KLH IgM molecules are thus sandwiched between immobilized KLH and the detection antibody conjugate. The wells are then washed to remove unbound HRP-labeled antibodies and TMB reagent is added and incubated for 20 minutes at room temperature. This results in the development of a blue color. Color development is stopped by the addition of stop solution, changing the color to yellow, and optical density is measured spectrophotometrically at 450 nm. The concentration of anti-KLH IgM is proportional to the optical density of the test sample.
Components:	Microtiter Plate: KLH coated 96-well plate (12 strips of 8 wells)

Product Details

	Enzyme Conjugate Solution: 11 mL Calibrator: Lyoph. Diluent Buffer (10x): 25 mL TMB Solution: 11 mL Stop Solution: 11 mL, 1N HCl Wash Buffer (20x): 50 mL.
Material not included:	Plate reader (450 nm) Micropipette and tips De-ionized water Graph paper (PC software is optional) Paper towels Polypropylene or glass tubes Vortex mixer Plate shaker/incubator Plate washer.

Target Details

Target:	KLH IgM
Target Type:	Antibody, Antibody

Application Details

Plate:	Pre-coated
Reagent Preparation:	Wash Buffer: The wash solution is provided as 20x stock. Prior to use dilute the contents of the bottle (50 mL) with 950 mL of distilled of deionized water. Diluent The diluent is provided as 10x stock. Prior to use estimate the final volume of diluent required for your assay and dilute one volume of the 10x stock with nine volumes of distilled or deionized water. Calibrator 1. The Rat anti-KLH IgM calibrator is provided as lyophilized stock. Reconstitute with 1 mL of distilled or deionized water. The reconstituted calibrator is stable at 4°C for one week but should be aliquoted and stored frozen at - 20°C after reconstitution if future use is intended. 2. Label 6 polypropylene or glass tubes as 250, 125, 62.5, 31.2, 15.6, and 7.8 ng/mL. 3. Into the tube labeled 250 ng/mL, pipette the volume of diluent detailed on the anti-KLH IgM calibration vial label. Then add the indicated volume of anti-KLH IgM calibrator (shown on the anti-KLH IgM calibrator vial label) and mix gently. This provides the 250 ng/mL calibrator. 4. Dispense 300 µL of diluent into the tubes labeled 125, 62.5, 31.2, 15.6, and 7.8 ng/mL.

	5. Prepare a 125 ng/mL calibrator by diluting and mixing 250 µL of the 250 ng/mL calibrator with 250 µL of diluent in the tube labelled 125 ng/mL. 6. Similarly prepare the 62.5, 31.25, 15.6, and 7.8 ng/mL calibrators by serial dilution.
Sample Preparation:	Note: Studies indicate that anti-KLH IgM is present in rat serum or plasma at concentrations ranging from approximately 20 to 200 µg/mL. In order to obtain values within range of the calibration curve, we suggest samples initially be diluted 1,000 fold using the following procedure for each sample tested. 1. Dispense 196 µL and 285 µL of diluent into separate tubes. 2. Pipette and mix 4 µL of the serum/plasma sample into the tube containing 196 µL of diluent. This provides a 50 fold diluted sample. 3. Mix 15 µL of the diluted sample with 285 µL of diluent in the second tube. This provides a 1,000 fold dilution of the sample. 4. Repeat this procedure for each sample to be tested. 5. Do not use dilutions lower than 500 fold.
Assay Procedure:	1. Secure the desired number of coated wells in the holder. 2. Dispense 100 µL of calibrators and diluted samples into the wells (we recommend that samples be tested in duplicate). 3. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 45 minutes. 4. Aspirate the contents of the microtiter wells and wash the wells 5 times with 1x wash solution using a plate washer (400 µL/well). The entire wash procedure should be performed as quickly as possible. 5. Strike the wells sharply onto absorbent paper or paper towels to remove all residual wash buffer. 6. Add 100 µL of enzyme conjugate reagent into each well. 7. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 45 minutes. 8. Wash as detailed in 4 and 5 above. 9. Dispense 100 µL of TMB reagent into each well. 10. Gently mix on an orbital micro-plate shaker at 100-150 rpm at room temperature for 20 minutes. 11. Stop the reaction by adding 100 µL of Stop Solution to each well. 12. Gently mix. It is important to make sure all the blue color changes to yellow. 13. Read the optical density at 450 nm with a microtiter plate reader within 5 minutes.
Calculation of Results:	1. Calculate the average absorbance values for each set of calibrators and samples.

Application Details

	2. Construct a calibration curve by plotting the mean absorbance obtained from each calibrator against its concentration in ng/mL on linear graph paper, with absorbance values on the vertical or Y axis and concentrations on the horizontal or X axis. 3. Using the mean absorbance value for each sample, determine the corresponding concentration of anti-KLH IgM in u/mL from the calibration curve. 4. Multiply the derived concentrations by the dilution factor to determine the actual concentration for anti-KLH IgM in the serum/plasma sample. 5. PC graphing software may be used for the above steps. 6. If the OD values of samples fall outside the calibration curve when tested at a dilution of 1,000, samples should be diluted appropriately and re-tested. Do not use dilutions lower than 500 fold.
Restrictions:	For Research Use only

Handling

Handling Advice:	Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of and in accordance with the instructions detailed above. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
Storage:	4 °C/-20 °C
Storage Comment:	Store at 4°C. Calibrators should be stored at -20°C for optimal stability. Microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air. The kit is stable until the expiration date when stored as noted in this section. General Instructions 1. Please read and understand the instructions thoroughly before using the kit. 2. This kit is designed to measure anti-KLH IgM levels in Rat serum or plasma collected 5 days after immunization with KLH. At this point the immune response originates almost exclusively from IgM. 3. All reagents should be allowed to reach room temperature (18-25°C) before use. 4. Samples should be diluted at least 500 fold in 1x diluent. 5. Optimum results are achieved if, at each step, reagents are pipetted into wells of the microtiter plate within 5 minutes.
Expiry Date:	The expiry date is stated on the label.